CLINICAL RESEARCH MEETING

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*The Clinical Correlation of In-Vitro Penicillin Sensitivity Tests**

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In-vitro penicillin sensitivity tests of organisms as a rule merely estimate the bacteriostatic activity of penicillin. The information gained from this type of test was not always helpful in establishing optimum dosage schedules for the treatment of diseases caused by penicillin inhibitable organisms. In actual practice, particularly in the treatment of subacute bacterial endocarditis, merely parallelling in the blood serum the in-vitro penicillin bacteriostatic activity did not consistently achieve effective therapeutic blood levels. As a result of clinical experience therefore the dictum was well founded that multiples of five to ten times the in-vitro sensitivity figure were essential for termination of the infection. It was subsequently suggested that "to be therapeutically effective, penicillin blood levels must be far in excess of the in-vitro bactericidal requirements" and that "inadequate dosage invites treatment failure and the organisms may acquire resistance that is so high as to render future therapeutic levels virtually unattainable."[1,2] It was early evident that there is both a bacteriostatic and a bactericidal zone of penicillin activity. While the arbitrary intensification of penicillin dosage in the treatment of subacute bacterial endocarditis was attended with greater clinical success, there were still sufficient treatment failures to justify intensive investigation for the causative factors. One result of this study was the discovery of a new type of non-hemolytic streptococcus, designated Streptococcus s.b.e.[3] The factors responsible for the refractory nature of this organism are under investigation and are not discernible in test tube studies per se. Laboratory techniques were devised to determine the bacteriostatic and minimal lethal dosage of penicillin for the infecting organisms. The routine use of these procedures has afforded an explanation for the failure of penicillin treatment with seemingly adequate dosage as judged by customary standards. Thus while the infecting non-hemolytic streptococcus of P.G., a patient with subacute bacterial endocarditis, had a bacteriostatic penicillin sensitivity of 0.008 Oxford units per cc. of test broth, a seven weeks span of therapy of 300,000 to 500,000 Oxford units of penicillin daily with average blood levels of 0.53 Oxford units per cc. failed to cure. A total of 22.4 million Oxford units was administered during this unsuccessful course of therapy. The answer was found in the determination of the minimum lethal dosage of penicillin for this causative organism, which proved to be 1 Oxford unit per cc. of test broth. When the daily penicillin dosage was lifted to 2 million Oxford units with resultant blood levels of 2 or more units per cc. of serum,

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the blood stream was sterilized and the infection was terminated. That subcurative doses of penicillin have a pronounced tendency to increase the resistance of infecting organisms was apparent in our series of 157 cases of subacute bacterial endocarditis treated with the combination of penicillin and heparin. This fundamental concept, the implications of which are now widely recognized, has been found to apply to infections in general.

REFERENCES

1. Loewe, L.: The Combined Use of Penicillin and Heparin in the Treatment of Subacute Bacterial Endocarditis, *Canad. Med. Assoc. Jour.*, 52:1, 1945.
2. Loewe, L.: The Combined Use of Anti-Infectives and Anti-coagulants in the Treatment of Subacute Bacterial Endocarditis, *Bull. N. Y. Acad. Med.*, 21:59, 1945.
3. Loewe, L., Plummer, N., Niven, C. F. Jr., Sherman, J. M.: Streptococcus S.B.E.: A Hitherto Undescribed Variety of Non-Hemolytic Streptococcus Recovered from Patients with Subacute Bacterial Endocarditis, *J.A.M.A.*, 130:257, 1946.

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Repository Injection of Penicillin in Water-in-Oil Emulsion

Effect on Early Syphilis—A Preliminary Report

ALFRED COHN, THEODORE ROSENTHAL and ISAAK GRUNSTEIN

In previous investigations a method was developed to cure gonorrhea by a single intramuscular injection of penicillin in water-in-oil emulsion. The administration of penicillin in such a vehicle has been now applied for the treatment of early syphilis. It is hoped that this method which reduces the number of daily intramuscular injections to one or two, may open the way for the ambulatory treatment of syphilitic patients with penicillin.

A total of 35 male patients with early syphilitic infections received daily injections of penicillin in water-in-oil emulsion over a period of 5 days. The daily dose ranged from 400,000 to 1,000,000 Oxford units of penicillin, given intramuscularly either at 12 or 24 hour intervals.

After total dosages of 2,000,000 to 5,000,000 Oxford units respectively, definite clinical regression of the syphilitic lesions were observed among all patients at the end of active therapy.

Darkfield examinations initially positive for Treponema pallidum, were found negative 24 hours after the initiation of treatment in all of 11 cases examined.

Complete healing of the primary chancre at the fifth day of treatment was noticed in 19 patients and healing within a period of 13 to 90 days took place in 13 cases. Of the remaining 3 patients one showed a clinical relapse when reëxamined 65 days after treatment, and the other 2 patients were delinquent.

Complete involution of the secondary manifestations immediately following treatment was observed among a group of 12 patients and within a period of 11 days to 6 weeks in the remaining 3 patients. Similar involution of lymphadenitis occurred either shortly after treatment or 5 to 7 months later.

Titrated Wassermann reactions and flocculation tests (Kahn, Kline and Mazzini) were done on the serum of each patient before treatment and repeated each time the patient reported for reëxamination. Four initially negative Wassermann sera remained negative during a period of 1 to 12 months. The positive Wassermann tests of 16 patients reversed to negative within a period of 5 to 28 weeks. The sera of 2 other patients yielded persistently positive Wassermann and flocculation tests over a period of 8 to 10 months, while their corresponding spinal fluid tests done 6 months after treatment, were negative.

The initial spinal fluid examinations of all patients before treatment were negative. The spinal fluid of 14 patients reëxamined 6 months after treatment were also negative.

Ten cases were followed for a period up to 6 months. In this group there occurred the only clinical relapse observed thus far, 60 days after treatment. This patient suffered from sero-positive primary syphilis and was treated over a period of 5 days with single daily injections of penicillin in water-in-oil emulsion (900,000 Oxford units

the first day and 700,000 Oxford units daily for 4 days), simultaneously with a daily dose of 100,000 Oxford units in saline.

Fourteen patients remain under observation for period of 6 to 10 months; none show any signs of clinical relapse; there are, however, 2 patients with persistent positive serologic reaction requiring further observation, and a third patient with secondary syphilis whose serologic test reversed from negative to positive 8 months after treatment.

Injections of penicillin in the oily vehicle did not produce any serious side effects except transitory pains of varying intensity at the site of injection. Herxheimer reactions of a mild degree occurred in most cases.

For purposes of comparison two patients were treated with corresponding single daily injections of penicillin (total dosages of 2,000,000 and 4,200,000 Oxford units) in saline alone; both patients suffered clinical relapses within 2 and 3 months respectively.

Comparison of penicillin serum and urine levels in patients receiving first a single injection of penicillin in saline and 5 days later a single injection of identical amounts of penicillin in water-in-oil emulsions shows the following findings:

1. No essential difference in the duration and height of penicillin serum levels was noticeable among the 2 groups.

2. The 24-hour urine levels of patients who received single injections of penicillin in water-in-oil emulsion were twenty to one thousand fold higher than the urine levels of the patients after single injections of identical amounts of penicillin in water.

3. These findings correlated with clinical results may indicate that the administration of penicillin in water-in-oil emulsion prolongs the therapeutic effect of the antibiotic agent within the body, even though assayable penicillin serum levels could not be detected consistently.

Four patients received oral penicillin treatment. Of three receiving 6,000,000 Oxford units over a period of 6 to 10 days, 2 remain under observation. They are completely negative both clinically and serologically for a period up to 10 months. The fourth patient received a total dosage of 12,000,000 Oxford units over a period of 10 days, and displayed a clinical relapse after 68 days.

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Galactose Removal Constant

*An Expression of Galactose Disappearance From the Blood Stream: Its Application As A Test For Liver Function**

HENRY COLCHER, ARTHUR J. PATEK, JR., AND FORREST E. KENDALL

The rate of disappearance of galactose from the blood stream after a rapid intravenous injection was studied in 64 patients with and without liver diseases. A 50 per cent galactose solution was injected intravenously in amounts corresponding to 0.5 gm. per kilo body weight. Blood specimens were obtained before and at 15 minute intervals after the injection for a period of 90 minutes. Urine was collected as voided over a 4-hour period. The determination of blood galactose was based upon Benedict's method for blood sugar after removal of glucose by fermentation according to the method of Raymond and Bianco.

When the logarithm of blood galactose concentration is plotted against time a straight line is obtained. This straight line may be interpreted to indicate that the rate of removal of galactose from the blood is directly proportional to the concentration in the blood. By use of the following equation a constant, K, was calculated.

$$K = \frac{2.3\ (\log C_1 - \log C_2)}{t_2 - t_1}$$

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C_1 and C_2 are the concentrations of blood galactose (in mg./100 cc.) at times, t_1 and t_2 expressed in minutes. K represents the fraction of the total amount of galactose present at any given time that is removed from the blood each minute. If K is multiplied by 100 the numerical value obtained expresses the percentage of the amount present that is removed each minute. This might be called the Galactose Removal Constant or G.R.C.

The Galactose Removal Constant can be calculated with sufficient accuracy from two blood specimens obtained at 15 and 45 minutes after intravenous injection. Under these conditions the constant may be calculated by the following equation:

$$\text{G.R.C.} = 7.6\ (\log \text{conc. at 15 min.} - \log \text{conc. at 45 min.})$$

The value of G.R.C. varied between 4.2 and 9.5 in 10 controls and was below 4 in 43 patients with parenchymal liver damage (26 patients with cirrhosis of the liver, 7 cases of hepatitis, and 10 patients with chronic passive congestion of the liver). In one patient with acute hepatitis, the value of G.R.C. was 1.9 and 1.94 on the 4th and 14th day of illness and returned to normal value of 6.2 three months after clinical recovery.

A series of determinations pertaining to liver functions were performed simultaneously with the galactose test on all patients. These included serum protein partitions, serum bilirubin, plasma prothrombin, cephalin cholesterol flocculation, bromsulphalein dye retention and urinary urobilinogen excretion. There was a good correlation between the values of galactose removal constant and other liver function tests.

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*The Etiologic Agent of Pemphigus Vulgaris**

ARTHUR W. GRACE

INTRODUCTION

In a search for additional data upon the etiologic agent of pemphigus vulgaris,[1,2] pathologic and control materials were inoculated into the brains of x-irradiated and normal mice.

MATERIALS

The inocula consisted of:

A. *Materials obtained from persons suffering from pemphigus vulgaris:*

Twelve blisters arising spontaneously; 3 cantharides-induced blisters; 8 cerebrospinal fluids; 6 whole bloods; 1 blood plasma; 5 blood sera; 2 washed blood cells; 3 tissues obtained at autopsy.

B. *Materials obtained from persons suffering from diseases other than pemphigus vulgaris:*

Three blisters arising spontaneously; 1 cantharides-induced blister.

C. *Materials obtained from normal persons:*

Three cantharides-induced blisters; 2 blood sera.

D. *Materials obtained from mice:*

Seventy-eight irradiated mouse brains containing heat-inactivated RP strain of virus; 15 irradiated normal mouse brains.

RESULTS

Nine strains of non-bacterial agents, transmissible to mice, were obtained from 7 persons suffering from pemphigus vulgaris. Four of the strains were derived from spontaneous blisters, 3 from cerebrospinal fluids and 2 from blood sera. No transmissible agents were obtained from any of the other materials. The strains were transmitted for the following number of passages respectively: 242, 11, 5, 4, 1, 1, 1, 1, 1. No attempt was made to transmit 3 of the ma-

* From the New York Hospital and the Department of Medicine, Cornell University Medical College and the Long Island College Hospital and the Department of Dermatology and Syphilology, Long Island College of Medicine. Aided by grants from the John and Mary R. Markle Foundation and from the Dazian Foundation for Medical Research.

terials for more than 1 passage.

The criterion of the presence of the agent was the appearance in the brains of infected mice of discrete and diffuse, macroscopic and microscopic, collections of polymorphonuclear leukocytes. In some instances, the spinal cord and meninges were also involved in the same histologic change. Sick animals whose brains did not show this change were not regarded as having been infected.

Close study was made of the RP strain of virus, which was transmitted for 242 passages, with the following results:

a. The virus is unlike any spontaneous mouse virus hitherto described.

b. Neutralization experiments were performed with sera obtained from 10 normal persons and from 13 persons suffering from pemphigus vulgaris. Some degree of neutralization was obtained with some normal sera but definitely higher degrees of (and in one case, complete) neutralization were obtained with pemphigus sera. The serum from another case of pemphigus showed the development of a high degree of neutralization coincident with clinical remission and its disappearance in a relapse which terminated in the death of the patient.

c. Rabbits received virus by corneal scarification and by intradermal, intracerebral, intratesticular and intraperitoneal routes. Guinea pigs were inoculated subcutaneously, intracerebrally and intraperitoneally. No pathologic changes were produced in the animals. The virus, therefore, was not that of herpes, vaccinia, rabies, choriomeningitis or lymphogranuloma venereum.

d. No growth occurred in medium used for the growth of pleuropneumonia-like organisms.

e. Experiments in which an inoculum was employed whose concentration was 20 times that used for infection of irradiated mice showed that, while infection of non-irradiated mice occurred readily, it was impossible to maintain the virus for more than 3 or 4 passages in non-irradiated mice.

f. Vesicles accompanied with intense ectodermal, mesodermal and entodermal cellular reaction were produced by depositing the virus on the chorioallantoic membrane of the developing chick.

CONCLUSION

The data gathered in this study warrant the conclusion that the virus whose characteristics are outlined in this paper is the etiologic agent of pemphigus vulgaris.

REFERENCES

1. An Agent, Transmissible to Mice, Obtained During a Study of Pemphigus Vulgaris. Grace, A. W., Suskind, F. H., *Proc. Soc. Exp. Biol. and Med.*, 1937, 37, 324.
2. An Investigation of the Etiology of Pemphigus Vulgaris, Grace, A. W., Suskind, F. H., *Jour. Invest. Dermat.*, 1939, 2, 1.

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Some Pharmacological and Clinical Experiences with Dimethylaminoethyl Benzhydryl Ether Hydrochloride (Benadryl)

THOMAS H. MCGAVACK, HERBERT ELIAS AND LINN J. BOYD

The action of dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl) was studied in 60 normal subjects and in more than 200 patients with various forms of allergic and other diseases in which sudden releases of histamine may play a pathogenetic role.

The various systems of the body were systematically checked in the 'normal' subjects and in some of the patients for both physiological and toxic actions of the drug.

The data obtained has been correlated under three main headings: (1) physiological and pharmacological effects of the drug as observed in "screening" tests for the functions of the various systems of the body in all of the 'normal' subjects and in some of the patients; (2) the therapeutic range of activity of benadryl; (3) the nature and frequency of toxic reactions.

1. *Effects of benadryl upon various bodily systems and functions.* The positive

data which were obtained in the study of 'normal' subjects and some of the patients indicated:

a. A depression of the secretion of gastric acid in 19 of 21 subjects studied, noticeable witn doses of 150 mg. daily, and occasionally reaching a state of complete suppression when 400 mg. were administered daily.

b. A depression of the dermal response to histamine in all of 20 normal subjects and in 7 patients studied. There was complete suppression of the response when patients were kept on from 300 to 400 mg. of drug daily for several weeks. Reactions to the injection of histamine were continuously abolished promptly by the intravenous administration of 20 mg. of benadryl.

c. An atropine-like action of topically applied benadryl upon the pupil of the eye, maximal in degree by the end of 1 hour. This was hastened not increased by epinephrine. Benadryl increased the action of atropine and decreased that of eserine.

d. The development of orthostatic hypotension in approximately 8 per cent of all subjects studied.

e. A moderate decrease in the capillary permeability of subjects receiving doses of 300 to 400 mg. of benadryl daily.

2. *The therapeutic range of activity of benadryl.* Benadryl proved to be most highly effective for the control of acute and chronic urticaria, angio-neurotic edema, allergic eczema, hay fever and vasomotor rhinitis. Good but less reliable effects were observed in patients with bronchial asthma, neurodermatitis, dysmenorrhea and spastic colon. In many instances relief was afforded patients who suffered from Ménière's syndrome, migraine, 'intractable' insomnia, a variety of gastrointestinal neuroses (other than spastic colon), and cardiac asthma. However, the results do not warrant conclusive statements regarding the routine usefulness of the drug in these conditions. A questionable influence upon essential hypertension and the tremor of paralysis agitans has been noted. In several other conditions of widely diverse nature, no beneficial action could be demonstrated.

3. *The nature and frequency of toxic reactions.* Drowsiness was the most frequent and most distressing untoward manifestation of the action of benadryl. It was usually most marked after the first dose or doses of the drug; later a "tolerance" was commonly established. A single dose of 50 mg. has evoked a severe response, necessitating the use of coffee or benzedrine sulfate to keep the subject awake. Other unpleasant symptoms in the order of their frequency included dizziness, blurring of vision, dryness of the mouth, and an "all gone feeling" at the pit of the stomach. None of the reactions observed in any way endangered the subject's life or health, and all disappeared in from one-half to several hours after discontinuing the drug. Moreover, as a rule the drug could be resumed without untoward effect by stepping up the dose gradually.

It was concluded that benadryl is a powerful antihistamine agent which has in addition weaker but definite antispasmodic activity of an atropine-like type.

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The Clinical Evolution of Vascular Damage in Diabetes Mellitus

HENRY DOLGER

The long recognized association of vascular damage with diabetes mellitus was emphasized in 1936 by Kimmelstiel and Wilson in a report on intercapillary glomerulosclerosis. Our observations indicate that this triad of retinopathy, hypertension, and albuminuria is more than a terminal "pathological" syndrome.

When diabetic patients, young and old, mild and severe, were scrutinized carefully over a period of years, such vascular damage was found in every instance. When 200 patients below fifty years of age were followed diligently for 25 years, not one escaped retinal hemorrhages, albuminuria and/or hypertension in varying degree. The

group investigated consisted of 16 whose age of onset of diabetes was below 10 years; 39 whose age of onset was between 10 and 20 years; 22 between 20 and 30 years; 43 between 30 and 40 years; and 80 between 40 and 50 years. There was no preëxisting hypertension in any of these persons.

Retinal hemorrhage was the predominant lesion when diligent search was made routinely, and it often preceded the appearance of albuminuria and/or hypertension. When this sequence was not observed it could probably be attributed to failure to notice transient hemorrhages which had resolved in the interval between examinations. All three lesions appeared in the patients regardless of the age of onset or degree of severity of the diabetes, the need for insulin, the type of diabetic "control" or diet employed, the blood cholesterol levels, or the absence of x-ray evidence of arterial calcification.

Careful repeated examinations of the eye grounds, blood pressure, and urine for albumin revealed *no* single instance where the diabetes had lasted 25 years in which the patient escaped the development of vascular damage. Some presented rapid progression into the full blown clinical syndrome of intercapillary glomerulosclerosis frequently with blindness. In the majority, however, the lesions progressed slowly with accelerated damage occasionally in isolated viscera and tissues such as renal, coronary and cerebral vessels, etc. The finding of specific renal vascular lesions on postmortem examination in 68 per cent of all diabetic patients was reported in 1944 by Lapply, Fitzes, and Dutra. This may now be considered a low estimate in view of our finding of vascular lesions in 100 per cent of all diabetics followed 25 years. In the middle age groups, the damage often appeared within 5 years of the onset of diabetes and not infrequently was noted coincidental with the onset. In a few instances, the lesion seemed to antedate the onset of clinically detectable diabetes.

Every diabetic would seem at present to be doomed to the inexorable development of vascular damage despite the benefit of insulin in prolonging life. At most, 25 years of freedom from arteriosclerosis can be offered even to the juvenile diabetic. The prevention of these inevitable sequelae are a challenge to the future.

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Insulin Resistance: A Case Study

THOMAS H. MCGAVACK, SOLOMON D. KLOTZ, MILDRED VOGEL *and* JAMES F. HART

A 64-year old man with diabetes of 7 years duration has been observed in 3 periods of "insulin resistance" with severe ketonuria and glycosuria, during which a "peak" daily dose of insulin has been 3250, 1320 (400 intravenously), and 500 units respectively. Between these periods the patient's urine has been free of sugar and acetone without exogenous insulin. During the third period of resistance, the presence of allergic antibodies has been demonstrated repeatedly by the use of the Kustner-Prausnitz method for the passive transfer of sensitivity. Such reactions disappeared within 10 weeks after insulin was discontinued and reappeared within 10 days after its resumption.

Other protective substances of a directly anti-insulin or anti-hypoglycemic nature were sought for in the blood of the patient. However, repeated tests failed to show that his blood was capable of protecting mice from otherwise fatal doses of insulin.

Following an acute pancreatitis associated with the formation of a pseudo-cyst, the patient's sensitivity to insulin has become normal, and the allergic reactions previously noted can no longer be demonstrated.

It is concluded that under certain circumstances insulin may act as an allergen; the development of antibodies may be so great that severe resistance to the action of insulin results. The data are discussed in relation to previous concepts of insulin resistance as revealed in the literature.

Experiences with Folic Acid in Macrocytic Anemia

LEO M. MEYER

Data are presented concerning the use of folic acid in the treatment of 7 cases of pernicious anemia. Three of the patients received folic acid in doses of 50 mg. orally or 20 mg. intramuscularly daily. Clinical improvement with relief of symptoms and increases in hemoglobin, red cells and reticulocytes followed. However, complete hematological remissions could not be obtained, and 2 of the patients showed progression of their neurological signs and symptoms. The third died on the 37th day of bronchopneumonia. Four other patients were treated with combinations of 5 or 10 mg. of folic acid plus ½ unit of liver extract intramuscularly, daily. On this regime symptoms were relieved and blood pictures became normal. Reticulocytes rose to levels greater than those anticipated with liver alone and 2 of the patients with neurological signs were distinctly improved.

The author concludes that folic acid, by itself, fails to raise the Hb and red cells to normal levels and is unable to prevent the development or progression of signs of subacute combined sclerosis. However, the combination folic acid and small doses of liver produces a more rapid rise in the Hb and RBC with higher reticulocytosis and is effective in preventing and curing involvement of the central nervous system.

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*A Study of Histochemically Demonstrable Liver Phosphatase in Experimental Obstructive Jaundice and in Human Postmortem Material**

M. WACHSTEIN and F. G. ZAK

Methods capable of demonstrating enzymatic reactions in tissue sections have opened a new approach to the study of various biological problems. In view of the marked changes in serum alkaline phosphatase activity occurring in liver disease, a study of this enzyme in liver sections under experimental conditions, as well as in postmortem material, was undertaken. Gomori's method, as modified by Kabat and Furth with some minor alterations was used.

Little stainable phosphatase was present in the livers of normal rats and mice. Increase in cytoplasmic alkaline phosphatase activity in the atrophic liver cells of starved, in the hydrophic liver cells of protein depleted mice, and to a lesser degree of rats, has been described in a previous paper (*Arch. Path.* 1945, *40*,57). In the livers of animals poisoned by phosphorus, chloroform and carbon-tetrachloride there was no significant increase of phosphatase in necrotic cells.

In continuation of these experiments the common bile duct was ligated in 9 dogs after an initial biopsy had been taken. In the dog liver the bile capillaries showed conspicuous phosphatase activity. After the ligation there occurred a marked rise in serum alkaline phosphatase as described by various investigators. There was marked dilatation of the bile capillaries, increasing with the duration of the experiment. This widening apparently was caused by the accumulation of phosphatase in the liver cells around these structures. Considerable dilatation of bile capillaries was also seen in the livers of several rabbits 4 to 5 days after the ligation of the common bile duct.

The postmortem material included both

* From the Laboratories of the E. A. Horton Memorial Hospital, Middletown, New York and Mt. Sinai Hospital, New York.

sections from livers not involved by disease, and sections from livers which had been the seat of various pathological processes. In liver sections from patients whose death was due to various causes and in which no significant gross or microscopic changes were found, only little alkaline phosphatase activity was demonstrable in the liver cells, while the sinusoids, as well as the bile capillaries, showed a varying degree of activity. In 8 cases of obstructive jaundice there was dilatation of bile capillaries which, however, was absent in the 2 remaining instances. The degree and extent of the widening of the bile capillaries as seen in these preparations stained for alkaline phosphatase activity varied considerably. It was only focal in some and fairly uniform in others. Necrotic liver cells in these livers as well as in a number of other livers in which extensive necrosis had occurred, did not show increase in enzymatic activity. In a case of subacute yellow atrophy and in a case of toxic (post-necrotic) cirrhosis, there was conspicuous activity in the proliferating connective tissue and the infiltrating round cells. Marked staining of the connective tissue occurred also in some cases of Laennec cirrhosis while in other cases, obviously in a more quiescent stage, the connective tissue showed only little activity. In several cases in which hepato-cellular changes were superimposed and in which jaundice as well as increase in serum alkaline phosphatase had been present, focal dilatation of bile capillaries was seen while otherwise the liver cells did not show any alteration in their enzymatic activity. In several livers of patients who died from various causes (cardiac failure, glomerulo-nephritis, etc.) but without clinical and anatomical evidence of significant liver disease, considerable increase in cytoplasmic alkaline phosphatase activity was found.

The origin of the increased serum alkaline phosphatase in liver damage is still controversial. The behavior of histochemically demonstrable phosphatase activity in the liver under experimental conditions and in human postmortem material favors the assumption that this rise is due not to increased production in the diseased liver, but rather to the inability of the liver cells to excrete the enzyme. This may be caused either by external obstruction, or by cellular dysfunction. The blood level of alkaline phosphatase probably is in addition influenced by extra-hepatic factors, since phosphatase can be excreted in the pancreatic juice and through the intestinal tract and in addition through the kidneys in some species. Disturbances in calcium and phosphor metabolism in hepatic disease may influence the production of this enzyme in the osseous system.

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